

21st March, 1952

Dear Lederberg,

Your typescript and your subsequent letter of March 10 have been received. I have had a talk with Hayes in London, and a few crosses have been going on in my absence, so that it will take me a long letter to deal with everything.

1) your typescript. I agree with it and have added a few notes to it on a separate sheet.

2) its publication. I doubt that we can publish exactly the same paper on two separate journals, but if one of the two journals were a bacteriological and the other a genetical one, it may be sufficient - and advantageous too - to write the same things in the different styles suitable for the two classes of readers. Hayes told me he would send a paper about his new findings, stating in it that he had heard of our previous work, and without pressing for publication. He is likely to publish on J. Gen. Micr., and we might send the European edition of our joint paper to the same journal, asking for its inclusion in the same issue as Hayes's paper. I am a member of the Soc. Gen. Micr. I shall write to Hayes about it as soon as I hear from you. Another, alternative, way of publishing in Europe would be on our Bollettino, again a bacteriological journal, which would certainly take a paper in English. As to the American edition, I should be very pleased if it were on Genetics, and I agree entirely about ~~authorships~~ authorships. Also, the idea of keeping further developments for future paper/s seems quite good to me, the effect on segregation of F+ (on which I also had some data, see later) and the relations between Hfr and F+ being some of the obvious ones. But a hint at some of such developments in the first paper/s might seem advisable.

3) Hayes. His work seems good, but he has undoubtedly messed the whole problem by drawing too simple conclusions and using improper terminology. His trouble is that he is not a geneticist. He thinks of "gametes" as of non-filterable phage particles. His streptomycin experiment was probably meant to show that "cell-free" suspensions (rather, suspensions free of living cells) but containing the phage particles able to effect the genetic transfer, would be fertile. When he heard of self-incompatibility he thought it should mean the loss of the self-reproducing phage-like gamete, and that this - and therefore fertility - could be reintroduced by infection. I have tried to explain to him the difference between a "gamete", representing the nucleus of a cell, whatever this gamete is, and the capacity of forming gametes, and have suggested to him a fuller use of the markers to get a clear cut difference between infection for F+, and ~~recombination~~ gamete formation. This advice has probably been unfortunate, since it turned out later that F+ affects segregation, and he may by now be confronted with a similar puzzle to the one that now confronts us, ~~with~~ However, I shall see him in September and have another discussion with him.

4) F+ effect on segregation. It is quite possible, as far as my data go, that the ~~whole~~ difference between BM and TLB₁ lines is wholly due to the F+ effect, as you say. My approach to the problem was a different one, as I was interested in mapping separately the BM and TLB₁ lines and compare their maps. Three allelic markers showed the same order in BM x BM or TLB₁ x TLB₁ crosses, but this is not the main thing. The puzzle is represented by the results of two crosses of type TLB₁-S^r sugars negative x TLB₁+ sugars positive, the formerly "forbidden" cross, now made possible by F+ transduction. Data on such a cross ~~with~~ in the coupling and repulsion phase in respect to: F+, i.e. F+ x F- and F- x F+ were made, and although giving the same (linear!) order, i.e. (TLB₁ ?) Ara Lac Gal Xyl Mal S

a b c d e f c.o. regions. →

the c.o. values are widely altered in the two crosses, apparently ~~with~~ according to some sort of gradient along the chromosome. Cross I is TLB_1-S^r sugars neg. $F^- \times TLB_1$ sugars + F^+ , Cross II is $F^+ \times F^-$. Triples, not given in detail, are roughly proportional to the expectations according to singles,

C.o.region	Cross I	Cross II	Cross III
a	1	1	2
b	73	15	50
c	31	5	32
d	109	120	27
e	31	97	24
f	16	80	5
triples	38	10	25

It thus seems that the position of F^+ in the cross affects pairing, or c.o. with some sort of gradient along the chromosome, ~~or~~ perhaps ~~by~~ reducing or increasing, either pairing or c.o. in one arm, but the coupling and repulsion effect of F^+ ~~would~~ indicates some asymmetry which is not easily explained. Perhaps F^+ determines the direction of the cross, in the sense that it determines formation of gametes, and these may not be representative of the whole nucleus but of a fraction of it, as if the chromosome had a tendency to get broken before the free end and thus lose some of the terminal markers, although the point of breakage may vary ~~from gamete to gamete~~ in various "male" gametes. Also, one might assume that F^+ gets a locus on the chromosome, and the strand carrying F^+ ~~has~~ is selected for. There is still ample room for imagination. It would be important to know what happens in a $F^+ \times F^+$ cross in the TLB_1 line. If, as you assume, only $F^+ \times F^-$ crosses are permissible, and some of the cells of an F^+ line are phenotypically F^- , then such a cross ~~would~~ should give data intermediate to those of crosses I and II. Cross III is a substitute for such an $F^+ \times F^+$ cross, in that there the TLB_1-S^r sugars negative F^+ strain was crossed to K-12. It does behave as somewhat intermediate between crosses I and II, and does not show major deviations from the linear order, pointing out that probably there is no major cytogenetic change in either BM and TLB_1 lines. A fourth cross, i.e. $TLB_1-S^r F^+ \times TLB_1 F^+$ will say much about it. It will soon be made, ~~as was~~ or rather repeated, as it was accidentally lost. I shall also be looking for the F^+/F^- C and R effect in the BM line, and checking some other points, i.e. whether independent F^+ strains which have got their F^+ by transduction, behave differently ~~order~~, (two independent ones behaved exactly alike); and also whether the origin of F^+ has any importance in it.

5) I shall be collecting some data on Hfr, and I hope you will do the same. It seems to me that it does affect the F^+ story to a large extent, Hfr being perhaps a mutant of the F^+ particle. The symbol F^h might in such a case be useful. After all, this would be merely a reversal of terminology, because, if I remember correctly our early correspondence, the first F^- strain you and I came across were called Ofr. Can you confirm that Hfr does not transduce F^+ in infection experiments, while it can do so in sexual propagation, and that some F^+ (transduced) TLB_1 - give a high frequency of recombination with Hfr, while others do not?

6) If you are looking for F- mutations in other lines, it may interest you that Hayes tested some 120 colonies from an F+ strain and found none F-. Why not try nitrogen mustard resistance? Out of three strains thus selected in 1949, one was Hfr, the other normal, the third F-.

7) I think it would help me in the work on isolation of F+ out of cells to have Maas's strain. Would it be possible, and shall I ask Dr. Maas directly.

8) Could you give me at your earliest convenience an answer on these two points. (i) Have you anything against me saying a few words about this F+ story at a local national congress of Microbiology. I am not keen myself about it, but have been asked to do so. Anyhow I can easily give it up at this stage. A short summary should be published in the proceedings, in Italian. Would you prefer coauthorship or your contribution being quoted at its full value in the text. I should not take this as the European edition of our joint paper, since the paper must be in Italian and is bound to be buried, in a highly condensed form, in the proceedings, which should have only local readers. The congress is to take place on April 15 and I should give the title and 10 lines of summary before April 5th or so, but the summary to be published (some two or three sheets) can go in later, so that you could well see it before it was sent for publication. I hope you will be entirely frank about it. (ii) I should like to quote your method and results with the replica-plating, which ~~seems to~~ ^{seems to} be the most convincing evidence against Hinshelwoodian objections. Could you let me have some more details about it: antibiotics used, bacteria, etc. I am expected to send the manuscript by the 15th of April.

Yours sincerely

Lawall

Notes on the paper : SELF-incompatibility in E. coli and genetic infection for fertility ?

N.B. I am not meaning that these things should be added, or added as such to the manuscript. They are just data, or considerations, relevant to the various items.

page 2, top. An independent occurrence of a BM-F- was found at Cambridge during selection for nitrogen mustard resistance, which implied a long exposure to the drug. The strain did not cross to TLB₁-, but it was found later that it would cross to other, non TLB₁ stocks and to filial TLB₁-.

Later, line 8. The B marker has apparently disappeared from most stocks which carried it at the beginning, presumably by back mutation and selective advantage of B+, so that all BM- stocks will grow with the addition of methionine only, and ~~xxx~~ the addition of B has no effect on back mutation on minimal of BM- strains. However, the M marker is an exceedingly stable one, and its back mutation can be secured only by the addition of limiting amounts of methionine (slightly more than 10⁻⁶) to the minimal medium, and, in some instances, with the additional help of UV. Three independent single-step reversions were obtained, called BM+. The crosses BM-S^r x BM+ (symbol B kept to indicate the origin of the strains) are always fertile, except that they need previous incubation ~~xxx~~ of the mixture in broth before plating on minimal St, in order to secure a decent amount of prototrophs. Incubation in a "rolling" apparatus (Ryan, MGB) determines an increased yield and shortens the necessary time, so that 3-5 hours are amply sufficient for a high yield, when starting from non-rolled ("depp"-grown cultures). Starting from rolled cultures there is almost no fertility (later, aeration effect).

Two independent reversions of TLB₁-, i.e. TLB₁+, obtained in three steps, were ~~xxx~~ tested ~~xxx~~ in crosses to (parental) TLB₁-S^r, and never was a single recombinant prototroph recovered; but a full yield was obtained when crossing to filial TLB₁-S^r. (five different filial strains tested, differing by recombined markers). Also, parental TLB₁-S^r crossed freely with filial TLB₁+ (prototrophs from BM- x TLB₁- cross; ~~carrying~~ eight different ones tested, carrying all the eight possible combinations of three markers).

It may be mentioned that the cross TLB₁-S^r x Pr, when fertile, does not require previous incubation of the mixture in broth, before plating on minimal St although incubation would increase the yield by a factor of 10x or more.

Heterothallism : see discussion .

Transmission of F+, page 3.

In bouillon, incubation at 37°, initial amounts of either F+ and F- types about 10⁷/ml. After 4^h, 13 out of 14 had become F+ ; after 8^h, 10 out of 16 ; after 24^h, 15/15 ; after 48^h 16/18. No exchange of markers observed in these strains to which F+ had been transduced. The transmission can occur also in the presence of streptomycin when the F+ donor strain is S^s; the yield is still high, but there was no ~~xxx~~ high bactericidal action of streptomycin in these conditions.

Adding raw DNA-ase to bouillon, then 4^h incubation of mixture: 14/36 infected. Controls without DNA-ase, 25/36.

Experiments with raw DNA-ase with and without citrate 1%. With citrate: transduced F+ 4 out of 7. Without citrate 1/8 (difference not significant).

In a similar experiment the treatments were started before mixing F+ and F- cells, and incubation together shortened to 3^h. Results with citrate 1/10 infected and without citrate 0/10. These experiments should better not be quoted until they will be repeated with purified DNA-ase, now available.

Trials to get F+ off cells :

filtering of broth or minimal cultures : ineffective.

grinding F+ cells with alumina ,with and without prior UV irradiation ,then filtering (membranefiltres): ineffective.

heating F+ cells at 60° half an hour, and also various other times and temperatures, then :

- 1) adding directly to F- cells: doubtful results (cultures not sterilised)
- 2) killing with chloroform, ineffective.
- 3) adding streptomycin (also after treatment at 508, a/2 hour) ineffective.

growing with citrate, or arsenate at various concns., then killing with chloroform : ineffective.

penicillin lysis, then filtering : ineffective.

Discussion.

1) inheritance of self-compatibility appears as extranuclear or as infective according to the angle under which it is looked at. The ménage à trois exp. might have been interpreted as due to 1) F+ hormone, 2) transduction ; but the fact that inheritance of F+ is extranuclear, as shown by filial strains, that filtrates are ineffective, that the infection is possible with a very high yield it seems that there is no need of assuming an F* hormone in addition to F+ infection.

2) nature of infective agent, ? DNA-ase experiment should, I think, be carried out in both laboratories, as it seems an essential point.

3) Are all filial stocks from an F- x F+ cross F+ ? If so, since transduction in minimal is limited, F+ transduction must play an essential role in the cross. Perhaps if DNA-ase sensitivity is confirmed, F- prototrophs might be obtained crossing in presence of DNA-ase.

4) However, the possibility of having cells with F- phenotype and F+ genotype (aeration, pantothenicless) shows that fertility and presence of F+ are not necessarily the same thing.

5) The problem of heterothallism is not settled. In your ^{last} letter you assume it possible that ~~only~~ some of the cells of an F+ stock are phenotypically F-, and that only F+ x F- crosses are permitted; then we are exactly in the same position as Paramecium, variety 1. The comparison of crosses I, II, III above seems to support to some extent the idea that only F+ x F- crosses are permissible.

6) Correlation of F+ with other effects :

- a) Hayes's effect I (St-resistance of crossability in an F+ strain). It may be worth seeing whether this effect is extended to all F- stocks. Also, if an exp where St-treated F+ x St-treated F+ is non-fertile (I asked Hayes ~~xxxxxxx~~ if he could do it) then it must be assumed that the contributions of the two parents are different, in the sense that there is anisogamy, a male-like and a female-like gamete (The latter may be the normal cell). It would also lead to conclude that only F+ x F- crosses are permissible, i.e. K-12 is heterothallic.

- b) Hayes's effect II (UV enhancement of ~~Fx~~ fertility) in an F+ strain). Again, it is possible that F+, Hayes's effect I (is it what you call G_±?) and Hayes's effect II may be the same thing but a more extensive testing of F+ and F- stocks would be needed.
- c) there may be a slight difference in cultural behaviour after UV of F+ and F- strains, the latter tending to form longer snakes (I am not quite sure of this).
- d) serological data. Spicer (unpublished) found a serological difference between W 677 and 58-161,
- e) smooth-rough or similar changes are not morphologically apparent, but Maccacaro (unpub.) finds that some F- strains (W 677 and related) are strongly agglutinated by NaCl 5% while some F+ are not. I have suggested that he tries acriflavine as well.
- f) would it not be better to give a hint that F+ affects segregations in some orderly, not fully explained way.